ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 37: 149–156 (2024)
DOI 10.3897/herpetozoa.37.e121610

First record of *Amolops truongi* Pham, Pham, Ngo, Sung, Ziegler & Le, 2023 (Anura, Ranidae) from China

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https://zoobank.org/EF4A72E6-8F1E-4E7D-9D92-EC764C872623

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Academic editor: Peter Mikulíček ♦ **Received** 25 February 2024 ♦ **Accepted** 16 April 2024 ♦ **Published** 29 May 2024

Abstract

We report the first record of *Amolops truongi* Pham, Pham, Ngo, Sung, Ziegler & Le, 2023 from China, based on four specimens collected in Yunnan Huanglianshan National Nature Reserve. This species was previously known only from the type locality in north-western Vietnam. Morphologically, the specimens from China correspond to the original description of *A. truongi* with a few variations and, phylogenetically, they are clustered with the type specimens of *A. truongi* from Vietnam with strong support. In this study, we also provide an updated diagnosis of this species combining the original description and new data.

Key Words

distribution, morphology, ND2, updated diagnosis, Yunnan Huanglianshan National Nature Reserve

Introduction

Amolops Cope, 1865 is a genus with a rapid increase in the number of species recently, making it the most speciose genus within the family Ranidae and having a wide distribution range from southern and eastern Himalayas to the Peninsular Malaysia (Dever et al. 2012; Pham et al. 2019; Wu et al. 2020; Mahony et al. 2022; Tang et al. 2023; Frost 2024; Li et al. 2024). Currently, 85 species are included in the genus (Frost 2024) and 52 have been recorded in China (AmphibiaChina 2024). However, it is important to note that the taxonomic systems of Frost (2024) and AmphibiaChina (2024) have different views on the validity of some species, thus not allowing a direct comparison of the total number of species in the genus *Amolops* and the number of species recorded in China.

Amolops truongi Pham, Pham, Ngo, Sung, Ziegler & Le, 2023 is a species which was originally discovered in Muong La District, Son La Province, north-western Vietnam (Pham et al. 2023). Currently, *A. truongi* is known only from the type locality (Pham et al. 2023).

During our herpetological expedition in Yunnan Huanglianshan National Nature Reserve, Yunnan Province, China, in 2023, we collected some specimens of *Amolops*. Morphological and molecular data show that four of them should be assigned to *A. truongi*. Herein, we report the distribution of this species in China for the first time, describe the specimens collected from China and provide an updated diagnosis of this species.

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Material and methods

The fieldwork was carried out under the permission of Yunnan Huanglianshan National Nature Reserve Management and Protection Bureau. Specimens were collected by hand at night. Liver tissues were stored in analytical pure ethanol and specimens were preserved in 75% ethanol. The newly-collected specimens were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Measurements were taken with a digital caliper to the nearest 0.1 mm. The methodology of measurements followed Pham et al. (2023) and the terminology describing the webbing formula followed Glaw and Vences (2007). The following morphological characteristics were recorded: snout-vent length (SVL), from the tip of the snout to the cloacal; head length (HL), from the back of the mandible to the tip of the snout; maximum head width (HW), across the angles of the jaws; snout length (SE), distance from the tip of the snout to the anterior corner of the eye; nostril to snout distance (SND), from the nostril to the tip of the snout; eye to nostril distance (END), from the anterior corner of the eye to the nostril; internarial distance (IND), the distance between nostrils; upper eyelid width (UEW), the maximum width of the upper eyelid; interorbital distance (IOD), the minimum distance between the upper eyelids; horizontal eye diameter (ED), from the anterior corner to the posterior corner of the eye; tympanum diameter (TD), the maximum diameter of the tympanum; tympanum-eye distance (TED), from the anterior margin of the tympanum to the posterior corner of the eye; fore-limb length (FLL), from the tip of the disc of the third finger to the axilla; finger III disc width (FTD), the maximum width of the disc of the third finger; hind-limb length (HLL), from the tip of the disc of the fourth toe to the groin; femur length (FL), from the vent to the knee; tibia length (TL), from the knee to the tarsus; foot and tarsus length (FOT), from the tarsus to the tip of the fourth toe; toe IV disc width (HTD), the maximum width of the disc of the fourth toe; inner metatarsal tubercle length (MTTi), the maximum length of the inner metatarsal tubercle.

A fragment of the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) was amplified via the polymerase chain reaction (PCR) using the primers Met-LND2: 5′–CAATGTTGGTTAAAATCCTTCC–3′ and Trp–HND2: 5′–AGGCTTTGAAGGCCTTTGGTC–3′ (Stuart et al. 2006). The experimental protocols of amplification and sequencing followed Pham et al. (2023). Sequences were assembled using SeqMan in Lasergene 7.1 (Burland 2000). All new sequences have been deposited in GenBank and additional ND2 sequences used in this study were also obtained from GenBank (Table 1).

The sequences were aligned using ClustalW (Thompson et al. 1994) integrated in MEGA X and the genetic divergences (uncorrected p-distance) were calculated in MEGA X (Kumar et al. 2018). The best substitution models GTR+F+I+G4 for Bayesian Inference and TIM+F+I+G4 for Maximum Likelihood phylogenetic analysis were selected using the Akaike Information Criterion (AIC) in ModelFinder (Kalyaanamoorthy et al. 2017). Maximum Likelihood phylogenetic analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015) with the nodal support estimated by 1,000 ultrafast bootstrap replicates. Bayesian Inference was performed in MrBayes 3.2.7 (Ronquist et al. 2012) and the Markov chains run for 1,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in and the remaining trees were used to estimate Bayesian posterior probabilities.

Results

The morphological measurements of the specimens from China are presented in Table 2. There is no significant difference in morphological characteristics between the specimens from China and the type specimens of *Amolops truongi* from Vietnam, except for minor variations. Genetically, Bayesian Inference and Maximum Likelihood phylogeny obtained the same topology, with slightly different supports for some nodes. The sequences of the specimens from China clustered with the type specimens of *A. truongi* from Vietnam with strong support (Fig. 1). The genetic distance (uncorrected p-distance) between the specimens from China and the type specimens of *A. truongi* was only 0.4% (Table 3).

Taxonomic account

Amolops truongi Pham, Pham, Ngo, Sung, Ziegler & Le, 2023

Figs 2, 3

Specimen examined. KIZ2023080–KIZ2023083, four adult males, all collected on 22 July 2023 by Shuo Liu from Qimaba Township, Luchun County, Honghe Prefecture, Yunnan Province, China (22°56'29"N, 102°6'52"E, elevation 1430 m a.s.l.).

Description of the specimens from China. Male body size relatively small, SVL 39.3–39.9 mm in adult males; head moderate long (HL/SVL 0.36–0.38), longer than wide (HL/HW 1.09–1.15); snout relatively long (SE/SVL 0.16), projecting beyond lower jaw; canthus rostralis distinct; loreal region concave; distance from nostril to snout tip equal to or slightly greater than distance from eye to nostril (SND/END 1.00–1.03); internarial distance greater than interorbital distance (IND/IOD 1.22–1.39); upper eyelid width narrower than interorbital distance (UEW/IOD 0.78–0.94); pupil oval, horizontal; tympanum distinct (TD/ED 0.35–0.40); tympanum-eye distance smaller than tympanum diameter (TED/TD 0.77–0.90); vomerine teeth present; choanae rounded; tongue cordiform, notched posteriorly; vocal sac opening on floor of mouth at corner, sac-like gular pouch, front margin positioned near to level of centre of orbit.

Table 1. Samples used for the phylogenetic analyses in this study.

Species	Voucher	Locality	Accession
Amolops adicola	BNHS 6121	Arunachal Pradesh, India	MZ231116
Amolops akhaorum	FMNH 271355	Luang Namtha, Laos	FJ417207
Amolops akhaorum	FMNH 271406	Luang Namtha, Laos	FJ417208
Amolops aniqiaoensis	SYNU04ll6016	Tibet, China	MN958715
Amolops aniqiaoensis	KIZ011136	Tibet, China	MN958717
Amolops archotaphus	CUMZ A 2000.62	Chiang Mai, Thailand	FJ417173
Amolops archotaphus	KIZ030888	Chiang Mai, Thailand	MN958719
Amolops cf. *bellulus*	KIZYPX9037	Yunnan, China	MN958723
Amolops cucae	AMNH 168727	Lao Cai, Vietnam	FJ417193
Amolops cucae	AMNH 168729	Lao Cai, Vietnam	FJ417194
Amolops compotrix	FMNH 256499	Khammouane, Laos	FJ417185
Amolops compotrix	FMNH 256500	Khammouane, Laos	FJ417190
Amolops chunganensis	KIZ03756	Hubei, China	MN958729
Amolops daorum	ROM 38501	Lao Cai, Vietnam	FJ417199
Amolops deng	KIZ014115	Tibet, China	MW111443
Amolops iriodes	AMNH 163926	Ha Giang, Vietnam	FJ417201
Amolops iriodes	AMNH 163928	Ha Giang, Vietnam	FJ417202
Amolops kohimaensis	WIIADA 751	Nagaland, India	MZ231118
Amolops mengdingensis	KIZ20160265	Yunnan, China	MK501814
Amolops mengdingensis	KIZ20160266	Yunnan, China	MK501815
Amolops monticola	WIIADA 544	Sikkim, India	MZ231117
Amolops nyingchiensis	KIZ012629	Tibet, China	MN958773
Amolops nyingchiensis	KIZ016416	Tibet, China	MW133377
Amolops putaoensis	GXNU W011	Kachin, Myanmar	MT901213
Amolops putaoensis	GXNU W005	Kachin, Myanmar	MT901214
Amolops truongi	IEBR 4995	Son La, Vietnam	OP157199
Amolops truongi	ZVNU.2022.01	Son La, Vietnam	OP157200
Amolops truongi	KIZ2023080	Yunnan, China	PP663261
Amolops truongi	KIZ2023081	Yunnan, China	PP663262
Amolops truongi	KIZ2023082	Yunnan, China	PP663263
Amolops truongi	KIZ2023083	Yunnan, China	PP663264
Amolops tuanjieensis	GXNU YU110003	Yunnan, China	MN832756
Amolops tuanjieensis	GXNU YU110006	Yunnan, China	MN832757
Amolops tuberodepressus	SCUM050433CHX	Yunnan, China	MN958786
Amolops viridimaculatus	KIZ048488	Yunnan, China	MN958789
Amolops vitreus	FMNH 258183	Phongsaly, Laos	FJ417212
Amolops vitreus	FMNH 258187	Phongsaly, Laos	FJ417213
Amolops wenshanensis	KU 292045	Guangxi, China	FJ417178
Amolops wenshanensis	KIZ021425	Yunnan, China	MG996763

Fore-limb moderate long (FLL/SVL 0.66–0.68); relative length of fingers III > IV > II > I; tips of outer three fingers expanded into discs with circum-marginal grooves; webbing between fingers absent; subarticular tubercles present, oval, formula 1, 1, 2, 2; supernumerary tubercles absent; inner metacarpal (thenar) tubercle large, oval; outer metacarpal tubercle indistinct; glandular nuptial pad on finger I.

Hind-limb relatively long (HLL/SVL 1.78–1.84); tibia longer than thigh length (TL/FL 1.08–1.12); relative length of toes IV > V > III > II > I; all toe tips expanded into discs; webbing between toes deeply incurved, webbing formula I0–1/2II0–1III0–1IV1–0V; subarticular tubercles distinct, oval, formula 1, 1, 2, 3, 2; inner metatarsal tubercle elongated; outer metatarsal tubercle absent.

Dorsal and lateral surface of head and body smooth with few very small tubercles present on temporal sides of head, above tibiae and vent; supratympanic fold indistinct; dorsolateral fold distinct; ventral surface smooth with flat tubercles on basal ventral thigh.

Colouration in life. Dorsal sides of head and body green or olive brown with some black dots; lateral side of head and tympanum dark brown or black; a white stripe extending from tip of snout to shoulder on each side; iris pale gold; flanks light brown or brown; dorsal surface of fore-limbs and hind-limbs light brown or brown with dark bands; throat, chest and belly cream with some brown dots; vocal sac orange or light yellow; ventral surface of fore-limbs light red or flesh-coloured; ventral surface of hind-limbs red flesh-coloured or flesh-coloured with some dark brown dots; toe webbing dark brown.

Updated diagnosis. SVL 37.5–41.3 mm in adult males, 61.5–62.5 mm in adult females; head moderate long (HL/SVL 0.35–0.38 in males, 0.35–0.36 in females), longer than wide; snout relatively long (SE/SVL 0.16 in males, 0.15 in females); vomerine teeth present; tympanum distinct, round (TD/ED 0.35–0.40 in males, 0.36–0.37 in females); skin smooth; supratympanic fold indistinct; dorsolateral fold present; fore-limb moderate long (FLL/SVL 0.65–0.72 in males, 0.64–0.66 in fe-

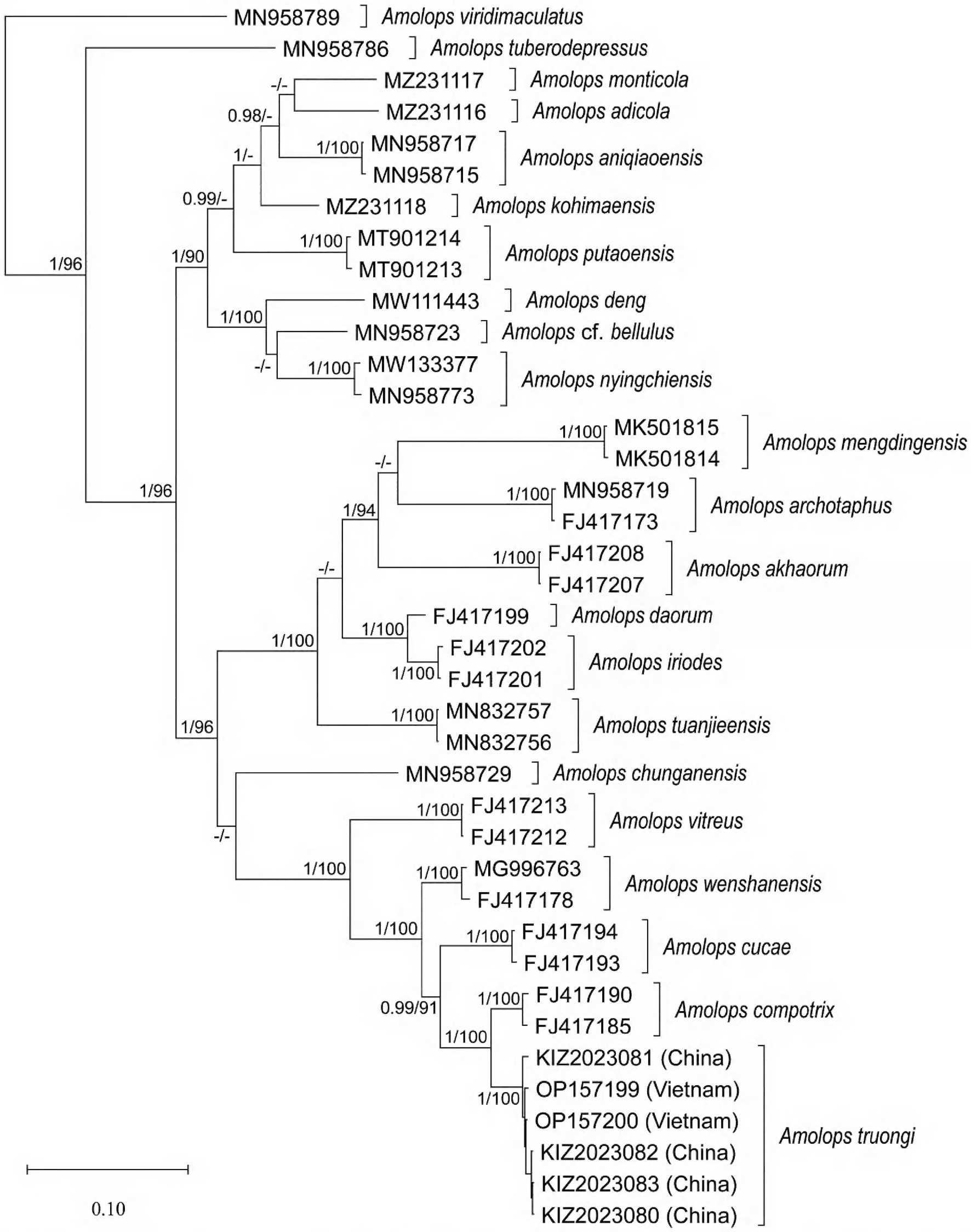

Figure 1. The Bayesian phylogenetic tree, based on the ND2 sequences. The numbers after and behind the "/" are the Bayesian posterior probabilities and the Maximum Likelihood ultrafast bootstrap values (> 0.90/90), respectively.

males); hind-limb relatively long (HLL/SVL 1.78–1.92 in males, 1.77–1.79 in females); webbing formula I0–1/2II0–1III0–1IV1–0V; external vocal sac present and finger I with nuptial pad in adult males.

Distribution. *Amolops truongi* is currently known from the type locality in Son La Province, north-western Vietnam and Lvchun County, Honghe Prefecture, southern Yunnan Province, China (Fig. 4).

Recommended common name. We suggest 山罗湍蛙 (Pinyin: shān luó tuān wā) as the Chinese name, deriving from the type locality Son La Province, Vietnam.

Figure 2. Dorsal view (top) and ventral view (bottom) of the specimens of *Amolops truongi* from China in preservative.

Discussion

The morphological characteristics of the specimens of *Amolops truongi* from China mostly agree with the original description by Pham et al. (2023), but there are also a few differences between them. The distance from the nostril to the snout tip is equal to or slightly greater than the distance from the eye to the nostril in the specimens from China, while the distance from the nostril to the snout tip is smaller than the distance from the eye to the nostril in the original description. The upper eyelid width is narrower than the interorbital distance in the specimens from China. while the upper eyelid width is wider than the interorbital distance in the original description. In addition, according to the original description of this species, the colour of the dorsum is light grey,

Table 2. Measurements (in mm) and proportions of the specimens of *Amolops truongi* from China (for abbreviations, see Material and methods).

	KIZ2023080	KIZ2023081	KIZ2023082	KIZ2023083
	Male	Male	Male	Male
SVL	39.4	39.7	39.3	39.9
HL	15.0	14.3	14.8	14.4
HW	13.3	13.1	13.4	12.5
SE	6.5	6.2	6.4	6.3
SND	3.4	3.4	3.2	3.2
END	3.4	3.3	3.2	3.2
IND	5.0	5.0	5.0	5.0
UEW	3.4	3.4	3.2	3.0
IOD	3.6	4.1	4.1	3.8
ED	5.6	5.4	5.7	5.3
TD	2.2	2.0	2.0	2.1
TED	1.7	1.8	1.7	1.7
FLL	26.1	27.0	26.2	26.5
FTD	1.9	2.0	1.8	1.9
HLL	70.8	72.9	70.0	71.6
FL	21.5	22.9	21.4	21.4
TL	23.6	24.7	23.9	23.8
FOT	32.2	32.4	31.9	32.4
HTD	1.6	1.6	1.6	1.5
MTTi	1.8	1.7	1.7	1.6
HL/SVL	0.38	0.36	0.38	0.36
HW/SVL	0.34	0.33	0.34	0.31
HL/HW	1.13	1.09	1.10	1.15
SE/SVL	0.16	0.16	0.16	0.16
SND/END	1.00	1.03	1.00	1.00
IND/IOD	1.39	1.22	1.22	1.32
UEW/IOD	0.94	0.83	0.78	0.79
ED/HL	0.37	0.38	0.39	0.37
ED/SE	0.86	0.87	0.89	0.84
TD/ED	0.39	0.37	0.35	0.40
TED/TD	0.77	0.90	0.85	0.81
FLL/SVL	0.66	0.68	0.67	0.66
HLL/SVL	1.80	1.84	1.78	1.79
TL/FL	1.10	1.08	1.12	1.11

while some specimens from China have a green dorsum. Therefore, based on these variants, we revised the diagnosis of this species.

Yunnan Huanglianshan National Nature Reserve is located in the south of Yunnan Province, it has a variety of terrain and vegetation types, which has long established its rich species diversity. In recent years, many new species have been discovered in this region, such as frogs *Leptobrachella aspera* Wang, Lyu, Qi & Wang, 2020 (Wang et al. 2020) and *Raorchestes huanglianshan* Jiang, Wang, Ren & Li, 2020 (Jiang et al. 2020) and plants *Agapetes heana* Y.H. Tong & J.D. Ya (Tong et al. 2021) and *Primula zhengyii* Bin Yang & Y.H. Tan (Yang et al. 2023). In addition, Yunnan Huanglianshan National Nature Reserve borders Vietnam in the south, so it is not surprising to find species that were previously considered to be distributed only in Vietnam. Previously, 52 species of the genus *Amolops* were recorded in China, 20 of which were distributed in Yunnan (Tang et al. 2023; Li et al. 2024). Our discovery of *A. truongi* in Yunnan increased the number of *Amolops* species in China to 53 and the number of *Amolops* species in Yunnan to 21.

Figure 3. Dorsal, lateral and ventral views of the male specimen KIZ2023081 (**A–C**) and dorsal, lateral and ventral views of the male specimen KIZ2023082 (**D–F**) of *Amolops truongi* from China in life.

Table 3. Uncorrected pairwise genetic distance (%) matrix, based on ND2 sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 *Amolops adicola*																				
2 *Amolops akhaorum*	17.4																			
3 *Amolops aniqiaoensis*	8.1	14.8																		
4 *Amolops archotaphus*	17.7	13.3	17.0																	
5 *Amolops* cf. *bellulus*	10.7	15.8	11.7	16.3																
6 *Amolops chunganensis*	13.5	14.6	13.5	15.8	11.8															
7 *Amolops compotrix*	15.1	17.3	15.3	18.8	15.7	15.3														
8 *Amolops cucae*	15.8	16.8	15.3	17.2	15.4	15.1	7.5													
9 *Amolops daorum*	16.8	12.1	15.9	12.3	15.1	13.7	17.0	17.6												
10 *Amolops deng*	12.9	15.9	13.3	17.0	8.4	13.9	16.8	15.3	14.1											
11 *Amolops iriodes*	17.1	12.4	15.7	13.1	15.1	13.6	17.0	17.3	2.8	14.3										
12 *Amolops kohimaensis*	7.0	15.6	6.6	16.3	9.4	13.3	14.9	14.8	14.6	10.4	14.9									
13 *Amolops mengdingensis*	18.6	14.1	16.2	13.4	17.0	16.1	18.3	17.9	13.7	17.2	14.1	17.1								
14 *Amolops monticola*	8.3	16.3	8.3	17.5	11.2	14.2	16.0	15.7	16.1	12.0	16.0	8.2	17.5							
15 *Amolops nyingchiensis*	13.1	16.5	12.0	16.8	7.7	13.8	16.2	16.2	14.7	8.6	14.4	10.5	17.5	11.5						
16 *Amolops putaoensis*	11.4	15.8	10.3	16.9	11.0	15.1	16.2	15.7	15.8	12.6	15.3	9.7	17.9	9.3	12.0					
17 *Amolops truongi* (China)	15.5	16.9	14.9	18.5	15.4	15.0	4.0	7.8	16.8	16.6	16.6	14.7	18.2	16.2	16.1	16.4				
18 *Amolops truongi* (Vietnam)	15.2	16.6	14.7	18.4	15.3	15.0	3.6	7.5	16.4	16.6	16.2	14.6	17.9	15.8	16.1	16.1	0.4			
19 *Amolops tuanjieensis*	17.2	13.1	15.1	13.0	15.4	14.2	16.5	16.5	9.5	15.0	10.3	13.9	15.0	14.5	14.5	14.8	16.9	16.8		
20 *Amolops vitreus*	14.4	15.6	14.6	16.9	14.7	13.9	11.1	12.1	16.0	14.8	16.1	13.4	18.6	14.0	15.7	14.6	12.0	11.5	14.8	
21 *Amolops wenshanensis*	15.0	15.5	14.3	16.6	15.1	13.6	6.8	6.7	15.8	15.7	15.3	14.0	17.1	13.9	14.8	14.7	7.1	6.8	14.5	10.2

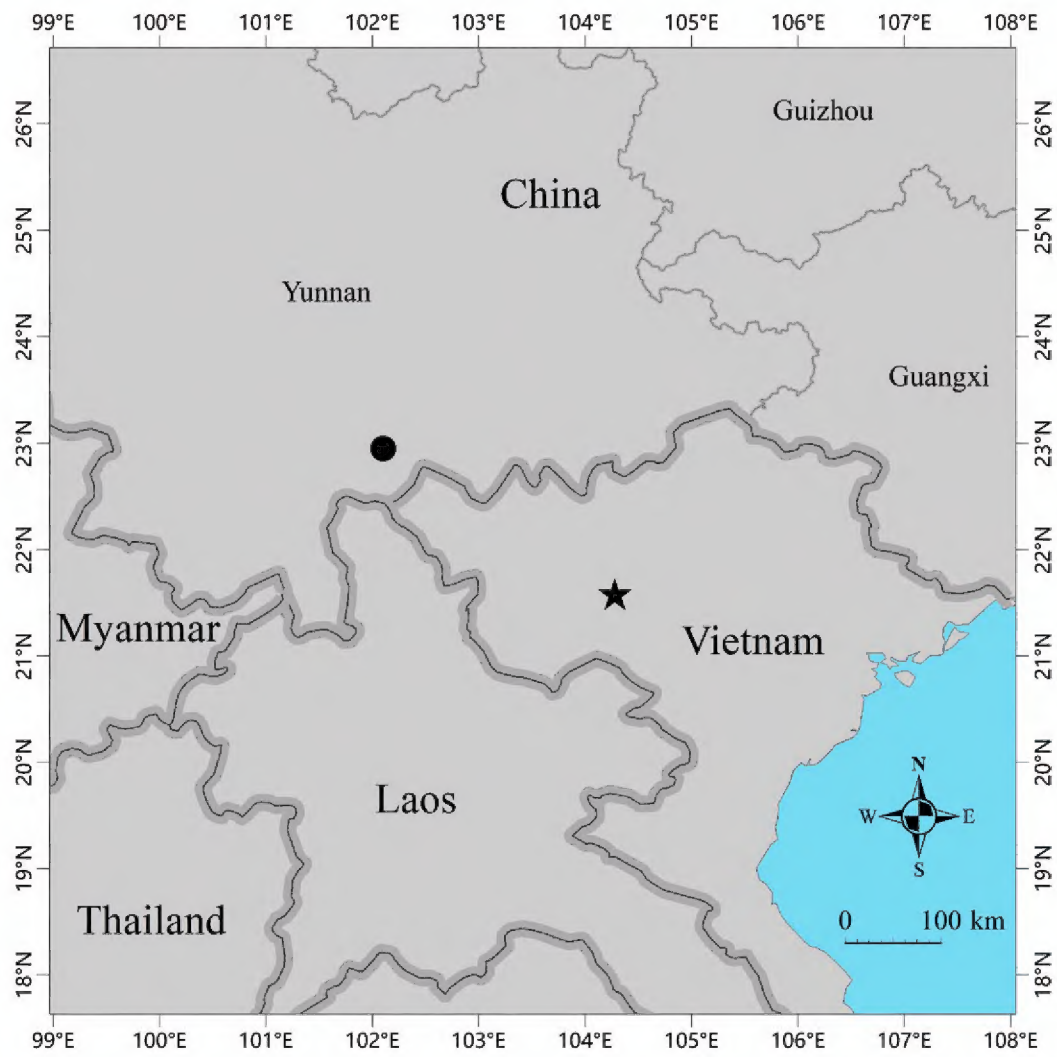

Figure 4. Map showing the type locality of *Amolops truongi* in north-western Vietnam (black star) and the new collection site from Lvchun County, Yunnan Province, China (black dot).

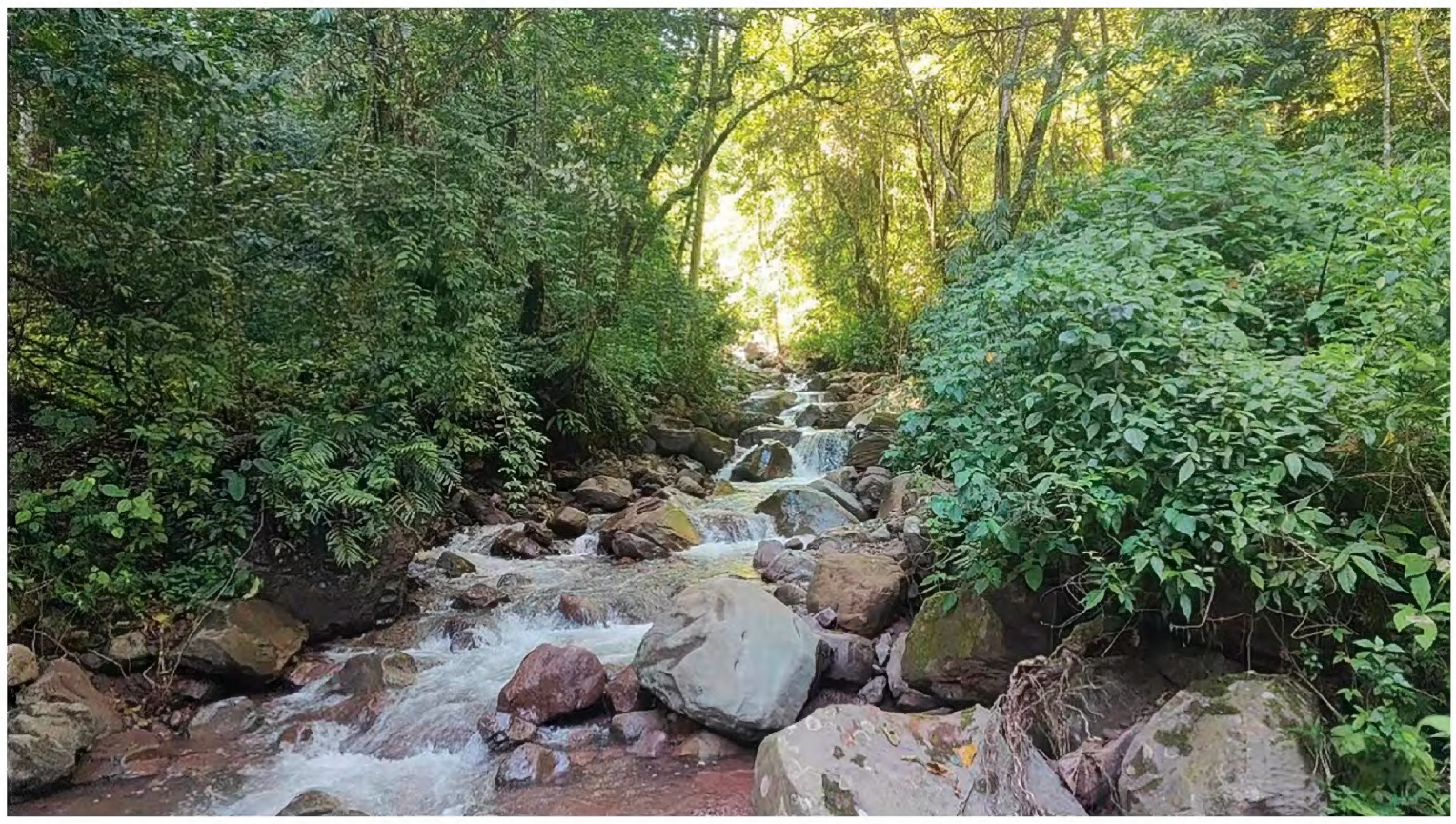

Figure 5. Habitat of the specimens of *Amolops truongi* collected in China.

Amolops truongi was previously known only from the type locality in north-western Vietnam (Pham et al. 2023). The new collection site in China extends the distribution range of this species to the northwest by approximately 290 km. The altitude of the type locality is 1360 m a.s.l., while the altitude of the new collection site in China is 1430 m a.s.l., the altitude difference between the two localities not being significant. According to Pham et al. (2023), the type specimens of this species were found on trees or limestone cliffs nearby a stream; however, the newly-collected specimens from China were all found on herbaceous plants nearby a river (Fig. 5), this being slightly different from those at the type locality. In addition, Pham et al. (2023) did not mention the courtship calls and breeding season of the species. During the surveys, we only collected males and did not find females or tadpoles and did not hear the courtship calls of males. Therefore, we are also unable to determine the breeding habits of this species. More field observations are needed to understand the habits and ecological information of *A. truongi*.

Acknowledgements

We thank the forest rangers of Yunnan Huanglianshan National Nature Reserve for their assistance in

the fieldwork. We would like to thank the editors and reviewers for their valuable comments on the manuscript. This work was supported by the Project of Huanglian Mountains National Nature Reserve Animal Diversity Expedition (Grant No. E2023HLS001), National Natural Science Foundation Project: Classificatory and Phylogenetic Studies on the *Amolops* frogs of China (Grant No. NSFC-31772424) and the project of the Ministry of Ecology and Environment of China: Investigation and assessment of amphibians and reptiles in southern Yunnan.

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